

* * * * * STN Columbus * * * * *

FILE 'HOME' ENTERED AT 14:59:34 ON 19 SEP 2003

=> fil .bec	SINCE FILE	TOTAL
COST IN U.S. DOLLARS	ENTRY	SESSION
FULL ESTIMATED COST	0.21	0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS, ESBIODBASE, BIOTECHNO, WPIDS' ENTERED AT 14:59:45 ON 19 SEP 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s alpha amylase#

FILE 'MEDLINE'

454272 ALPHA
20114 AMYLASE#
L1 4463 ALPHA AMYLASE#
(ALPHA(W) AMYLASE#)

FILE 'SCISEARCH'

642997 ALPHA
16176 AMYLASE#
L2 7215 ALPHA AMYLASE#
(ALPHA(W) AMYLASE#)

FILE 'LIFESCI'

147374 "ALPHA"
4283 AMYLASE#
L3 2572 ALPHA AMYLASE#
("ALPHA"(W) AMYLASE#)

FILE 'BIOTECHDS'

23979 ALPHA
4870 AMYLASE#
L4 3195 ALPHA AMYLASE#
(ALPHA(W) AMYLASE#)

FILE 'BIOSIS'

596697 ALPHA
26984 AMYLASE#
L5 9606 ALPHA AMYLASE#
(ALPHA(W) AMYLASE#)

FILE 'EMBASE'

508813 "ALPHA"
14781 AMYLASE#
L6 3284 ALPHA AMYLASE#
("ALPHA"(W) AMYLASE#)

FILE 'HCAPLUS'

1434449 ALPHA
42789 AMYLASE#
L7 17646 ALPHA AMYLASE#
(ALPHA(W) AMYLASE#)

FILE 'NTIS'

28426 ALPHA
163 AMYLASE#
L8 60 ALPHA AMYLASE#
(ALPHA(W) AMYLASE#)

FILE 'ESBIOBASE'
176805 ALPHA
3749 AMYLASE#
L9 1783 ALPHA AMYLASE#
(ALPHA (W) AMYLASE#)

FILE 'BIOTECHNO'
183303 ALPHA
4113 AMYLASE#
L10 2087 ALPHA AMYLASE#
(ALPHA (W) AMYLASE#)

FILE 'WPIDS'
164909 ALPHA
5095 AMYLASE#
L11 2102 ALPHA AMYLASE#
(ALPHA (W) AMYLASE#)

TOTAL FOR ALL FILES
L12 54013 ALPHA AMYLASE#

=> s l12(5a)gene/q
FILE 'MEDLINE'
L13 568 L1 (5A) GENE/Q

FILE 'SCISEARCH'
L14 808 L2 (5A) GENE/Q

FILE 'LIFESCI'
L15 578 L3 (5A) GENE/Q

FILE 'BIOTECHDS'
L16 732 L4 (5A) GENE/Q

FILE 'BIOSIS'
L17 1003 L5 (5A) GENE/Q

FILE 'EMBASE'
L18 446 L6 (5A) GENE/Q

FILE 'HCAPLUS'
L19 1713 L7 (5A) GENE/Q

FILE 'NTIS'
L20 5 L8 (5A) GENE/Q

FILE 'ESBIOBASE'
L21 273 L9 (5A) GENE/Q

FILE 'BIOTECHNO'
L22 495 L10 (5A) GENE/Q

FILE 'WPIDS'
L23 156 L11 (5A) GENE/Q

TOTAL FOR ALL FILES
L24 6777 L12 (5A) GENE/Q

=> s hyperthermophil? or thermophil?
FILE 'MEDLINE'
1419 HYPERTHERMOPHIL?
7477 THERMOPHIL?
L25 8631 HYPERTHERMOPHIL? OR THERMOPHIL?

FILE 'SCISEARCH'
2090 HYPERTHERMOPHIL?
12850 THERMOPHIL?
L26 14345 HYPERTHERMOPHIL? OR THERMOPHIL?

FILE 'LIFESCI'
1155 HYPERTHERMOPHIL?
7671 THERMOPHIL?
L27 8294 HYPERTHERMOPHIL? OR THERMOPHIL?

FILE 'BIOTECHDS'
282 HYPERTHERMOPHIL?
5266 THERMOPHIL?
L28 5325 HYPERTHERMOPHIL? OR THERMOPHIL?

FILE 'BIOSIS'
1834 HYPERTHERMOPHIL?
16462 THERMOPHIL?
L29 17388 HYPERTHERMOPHIL? OR THERMOPHIL?

FILE 'EMBASE'
1374 HYPERTHERMOPHIL?
8071 THERMOPHIL?
L30 8712 HYPERTHERMOPHIL? OR THERMOPHIL?

FILE 'HCAPLUS'
1988 HYPERTHERMOPHIL?
17169 THERMOPHIL?
L31 18713 HYPERTHERMOPHIL? OR THERMOPHIL?

FILE 'NTIS'
31 HYPERTHERMOPHIL?
485 THERMOPHIL?
L32 505 HYPERTHERMOPHIL? OR THERMOPHIL?

FILE 'ESBIOBASE'
1267 HYPERTHERMOPHIL?
4548 THERMOPHIL?
L33 5543 HYPERTHERMOPHIL? OR THERMOPHIL?

FILE 'BIOTECHNO'
1252 HYPERTHERMOPHIL?
6720 THERMOPHIL?
L34 7311 HYPERTHERMOPHIL? OR THERMOPHIL?

FILE 'WPIDS'
47 HYPERTHERMOPHIL?
1931 THERMOPHIL?
L35 1956 HYPERTHERMOPHIL? OR THERMOPHIL?

TOTAL FOR ALL FILES
L36 96723 HYPERTHERMOPHIL? OR THERMOPHIL?

=> s 124 and 136
FILE 'MEDLINE'
L37 29 L13 AND L25

FILE 'SCISEARCH'
L38 36 L14 AND L26

FILE 'LIFESCI'
L39 37 L15 AND L27

FILE 'BIOTECHDS'

L40 94 L16 AND L28

FILE 'BIOSIS'

L41 46 L17 AND L29

FILE 'EMBASE'

L42 39 L18 AND L30

FILE 'HCAPLUS'

L43 67 L19 AND L31

FILE 'NTIS'

L44 1 L20 AND L32

FILE 'ESBIOBASE'

L45 25 L21 AND L33

FILE 'BIOTECHNO'

L46 40 L22 AND L34

FILE 'WPIDS'

L47 8 L23 AND L35

TOTAL FOR ALL FILES

L48 422 L24 AND L36

=> s l48 not 2002-2003/py

FILE 'MEDLINE'

897062 2002-2003/PY

L49 26 L37 NOT 2002-2003/PY

FILE 'SCISEARCH'

1610344 2002-2003/PY

L50 33 L38 NOT 2002-2003/PY

FILE 'LIFESCI'

137782 2002-2003/PY

L51 32 L39 NOT 2002-2003/PY

FILE 'BIOTECHDS'

36013 2002-2003/PY

L52 90 L40 NOT 2002-2003/PY

FILE 'BIOSIS'

812724 2002-2003/PY

L53 40 L41 NOT 2002-2003/PY

FILE 'EMBASE'

739028 2002-2003/PY

L54 34 L42 NOT 2002-2003/PY

FILE 'HCAPLUS'

1760625 2002-2003/PY

L55 58 L43 NOT 2002-2003/PY

FILE 'NTIS'

17588 2002-2003/PY

L56 1 L44 NOT 2002-2003/PY

FILE 'ESBIOBASE'

467486 2002-2003/PY

L57 21 L45 NOT 2002-2003/PY

FILE 'BIOTECHNO'

203275 2002-2003/PY
L58 34 L46 NOT 2002-2003/PY

FILE 'WPIDS'
1725290 2002-2003/PY
L59 6 L47 NOT 2002-2003/PY

TOTAL FOR ALL FILES
L60 375 L48 NOT 2002-2003/PY

=> dup rem l60
PROCESSING COMPLETED FOR L60
L61 134 DUP REM L60 (241 DUPLICATES REMOVED)

=> d tot

L61 ANSWER 1 OF 134 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Novel, thermostable family-13-like glycoside hydrolase from *Methanococcus jannaschii*
SO *Folia Microbiologica* (Prague, Czech Republic) (2001), 46(6), 475-481
CODEN: FOMIAZ; ISSN: 0015-5632
AU Kim, J.-W.; Terc, H. A.; Flowers, L. O.; Whiteley, M.; Peeples, T. L.
AN 2002:140158 HCAPLUS
DN 137:59380

L61 ANSWER 2 OF 134 MEDLINE on STN DUPLICATE 1
TI Biochemical confirmation and characterization of the family-57-like alpha-amylase of *Methanococcus jannaschii*.
SO *FOLIA MICROBIOLOGICA*, (2001) 46 (6) 467-73.
Journal code: 0376757. ISSN: 0015-5632.
AU Kim J W; Flowers L O; Whiteley M; Peeples T L
AN 2002167588 MEDLINE

L61 ANSWER 3 OF 134 MEDLINE on STN DUPLICATE 2
TI Novel glucoamylase-type enzymes from *Thermoactinomyces vulgaris* and *Methanococcus jannaschii* whose genes are found in the flanking region of the **alpha-amylase genes**.
SO *APPLIED MICROBIOLOGY AND BIOTECHNOLOGY*, (2001 Aug) 56 (3-4) 465-73.
Journal code: 8406612. ISSN: 0175-7598.
AU Uotsu-Tomita R; Tonozuka T; Sakai H; Sakano Y
AN 2001499375 MEDLINE

L61 ANSWER 4 OF 134 MEDLINE on STN DUPLICATE 3
TI Cloning and expression of alpha-amylase from the **hyperthermophilic** archaeon *Pyrococcus woesei* in the moderately halophilic bacterium *Halomonas elongata*.
SO *JOURNAL OF APPLIED MICROBIOLOGY*, (2000 Mar) 88 (3) 495-503.
Journal code: 9706280. ISSN: 1364-5072.
AU Frillingos S; Linden A; Niehaus F; Vargas C; Nieto J J; Ventosa A; Antranikian G; Drainas C
AN 2000212011 MEDLINE

L61 ANSWER 5 OF 134 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
TI Cloning and expression of the **gene** encoding novel **alpha**-**amylase** from *Sulfolobus shibatae* in *E. coli*.
SO Weishengwu Xuebao, (June, 2000) Vol. 40, No. 3, pp. 323-326. print.
ISSN: 0001-6209.
AU Liu Li (1); Chen Wei (1); Jin Cheng
AN 2001:49285 BIOSIS

L61 ANSWER 6 OF 134 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Characterization of hygromycin-resistant transformants of **thermophilic** fungus *Thermomyces lanuginosus*; plasmid pMP6-mediated hygromycin-resistance **gene** transfer

- together with **alpha-amylase**, glucoamylase,
polygalacturonase and endo-1,4-beta-D-xylanase production
- SO World J.Microbiol.Biotechnol.; (2000) 16, 3, 303-06
CODEN: WJMBEY ISSN: 0959-3993
- AU Chadha B S; Kaur R; Saini H S; Singh S
AN 2000-10928 BIOTECHDS
- L61 ANSWER 7 OF 134 MEDLINE on STN DUPLICATE 4
TI Single-step purification of a recombinant thermostable alpha-amylase after
solubilization of the enzyme from insoluble aggregates.
- SO JOURNAL OF CHROMATOGRAPHY. B, BIOMEDICAL SCIENCES AND APPLICATIONS, (2000
Jan 14) 737 (1-2) 253-9.
Journal code: 9714109. ISSN: 1387-2273.
- AU Linden A; Niehaus F; Antranikian G
AN 2000143297 MEDLINE
- L61 ANSWER 8 OF 134 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
TI Releasing profiles of gene products from recombinant Escherichia coli in a
high-voltage pulsed electric field
- SO BIOCHEMICAL ENGINEERING JOURNAL, (JUN 2000) Vol. 5, No. 2, pp. 149-155.
Publisher: ELSEVIER SCIENCE SA, PO BOX 564, 1001 LAUSANNE, SWITZERLAND.
ISSN: 1369-703X.
- AU Ohshima T (Reprint); Hama Y; Sato M
AN 2000:414547 SCISEARCH
- L61 ANSWER 9 OF 134 MEDLINE on STN DUPLICATE 5
TI Cloning and expression of an **alpha-amylase** encoding
gene from the **hyperthermophilic** archaeobacterium
Thermococcus hydrothermalis and biochemical characterisation of the
recombinant enzyme.
- SO FEMS MICROBIOLOGY LETTERS, (2000 May 1) 186 (1) 67-71.
Journal code: 7705721. ISSN: 0378-1097.
- AU Leveque E; Haye B; Belarbi A
AN 2000395702 MEDLINE
- L61 ANSWER 10 OF 134 MEDLINE on STN DUPLICATE 6
TI Engineering direct fructose production in processed potato tubers by
expressing a bifunctional **alpha-amylase**/glucose
isomerase **gene** complex.
- SO BIOTECHNOLOGY AND BIOENGINEERING, (2000 Oct 5) 70 (1) 9-16.
Journal code: 7502021. ISSN: 0006-3592.
- AU Beaujean A; Ducrocq-Assaf C; Sangwan R S; Lilius G; Bulow L;
Sangwan-Norreel B S
AN 2001028442 MEDLINE
- L61 ANSWER 11 OF 134 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Production of recombinant **thermophilic** alpha-amylase activity
at low pH;
Thermococcus hydrothermalis recombinant enzyme production via plasmid
pEAMY101 and plasmid p662EL100 expression in Escherichia coli
- AU Leveque E; Belarbi A; Haye B
AN 2000-03337 BIOTECHDS
PI FR 2778412 12 Nov 1999
- L61 ANSWER 12 OF 134 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
TI A unique chitinase with dual active sites and triple substrate binding
sites from the **hyperthermophilic** archaeon Pyrococcus
kodakaraensis KOD1
- SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (DEC 1999) Vol. 65, No. 12, pp.
5338-5344.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW,
WASHINGTON, DC 20005-4171.
ISSN: 0099-2240.
- AU Tanaka T; Fujiwara S; Nishikori S; Fukui T; Takagi M; Imanaka T (Reprint)

AN 1999:949061 SCISEARCH

L61 ANSWER 13 OF 134 HCAPLUS COPYRIGHT 2003 ACS on STN
 TI Coordinate transcriptional control in the **hyperthermophilic** archaeon *Sulfolobus solfataricus*
 SO Journal of Bacteriology (1999), 181(13), 3920-3927
 CODEN: JOBAAY; ISSN: 0021-9193
 AU Haseltine, Cynthia; Montalvo-Rodriguez, Rafael; Bini, Elisabetta; Carl, Audrey; Blum, Paul
 AN 1999:414107 HCAPLUS
 DN 131:195320

L61 ANSWER 14 OF 134 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
 TI Purification and characterization of an extremely thermostable cyclomaltodextrin glucanotransferase from a newly isolated **hyperthermophilic** archaeon, a *Thermococcus* sp.
 SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (MAY 1999) Vol. 65, No. 5, pp. 1991-1997.
 Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
 ISSN: 0099-2240.
 AU Tachibana Y (Reprint); Kuramura A; Shirasaka N; Suzuki Y; Yamamoto T; Fujiwara S; Takagi M; Imanaka T
 AN 1999:353855 SCISEARCH

L61 ANSWER 15 OF 134 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 TI Extragenic pleiotropic mutations that repress glycosyl hydrolase expression in the **hyperthermophilic** archaeon *Sulfolobus solfataricus*.
 SO Genetics, (Aug., 1999) Vol. 152, No. 4, pp. 1353-1361.
 ISSN: 0016-6731.
 AU Haseltine, Cynthia; Montalvo-Rodriguez, Rafael; Carl, Audrey; Bini, Elisabetta; Blum, Paul (1)
 AN 1999:416085 BIOSIS

L61 ANSWER 16 OF 134 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
 TI Fermentation of starch by *Klebsiella oxytoca* P2, containing plasmids with **alpha-amylase** and pullulanase **genes**; for use in enzyme production or ethanol production
 SO Biotechnol.Bioeng.; (1999) 65, 6, 673-76
 CODEN: BIBIAU ISSN: 0006-3592
 AU dos Santos V L; Fernandes Araujo E; Goncalves de Barros E; Vieira Guimaraes W
 AN 2000-00826 BIOTECHDS

L61 ANSWER 17 OF 134 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
 TI Production of alpha-amylase in fed-batch cultures of vgb+ and vbg-recombinant *Escherichia coli*: some observations; *Bacillus stearothermophilus* recombinant enzyme production using host expressing (or not expressing) *Vitreoscilla* hemoglobin
 SO Biotechnol.Prog.; (1999) 15, 4, 640-45
 CODEN: BIPRET ISSN: 8756-7938
 AU Enayati N; Tari C; *Parulekar S J; Stark B C; Webster D A
 AN 1999-11780 BIOTECHDS

L61 ANSWER 18 OF 134 HCAPLUS COPYRIGHT 2003 ACS on STN
 TI Purification and characterization of the heat-labile .alpha.-amylase secreted by the psychrophilic bacterium TAC 240B
 SO Canadian Journal of Microbiology (1999), 45(6), 452-457
 CODEN: CJMIAZ; ISSN: 0008-4166
 AU Chessa, Jean-Pierre; Feller, Georges; Gerday, Charles
 AN 1999:504065 HCAPLUS
 DN 131:254106

- L61 ANSWER 19 OF 134 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 TI Close evolutionary relatedness of alpha-amylases from Archaea and plants.
 SO Journal of Molecular Evolution, (April, 1999) Vol. 48, No. 4, pp. 421-426.
 ISSN: 0022-2844.
 AU Janecek, Stefan (1); Leveque, Emmanuel; Belarbi, Abdel; Haye, Bernard
 AN 1999:220372 BIOSIS
- L61 ANSWER 20 OF 134 HCAPLUS COPYRIGHT 2003 ACS on STN
 TI Bioengineering of amylase and xylose isomerase thermozymes
 SO Special Publication - Royal Society of Chemistry (1999), 246(Recent
 Advances in Carbohydrate Bioengineering), 253-262
 CODEN: SROCDQ; ISSN: 0260-6291
 AU Zeikus, J. G.; Savchenko, A.; Sriprapundh, D.; Vieille, Claire
 AN 2000:29433 HCAPLUS
 DN 132:193269
- L61 ANSWER 21 OF 134 HCAPLUS COPYRIGHT 2003 ACS on STN
 TI Amylase and 16S rRNA genes from a **hyperthermophilic**
 archaeobacterium
 SO Journal of Applied Microbiology (1999), 86(1), 93-107
 CODEN: JAMIFK; ISSN: 1364-5072
 AU Jones, R. A.; Jermin, L. S.; Easteal, S.; Patel, B. K. C.; Beacham, I. R.
 AN 1999:138511 HCAPLUS
 DN 131:40353
- L61 ANSWER 22 OF 134 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
 TI Regulation of the expression of amy T01 encoding a thermostable
 alpha-amylase from Streptomyces sp. T01, in its original host and in
 Streptomyces lividans TK24;
 thermostable alpha-amylase production
 SO FEMS Microbiol.Lett.; (1999) 181, 1, 31-39
 CODEN: FMLED7 ISSN: 0378-1097
 AU Mellouli L; Guerineau M; Bejar S; Virolle M J
 AN 2000-00399 BIOTECHDS
- L61 ANSWER 23 OF 134 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
 TI Synthetic and excretion of alpha-amylase in vgb+ and vgb-recombinant
 Escherichia coli: a comparative study;
 Bacillus stearothermophilus recombinant alpha-amylase production
 SO Biotechnol.Bioeng.; (1998) 59, 6, 673-78
 CODEN: BIBIAU ISSN: 0006-3592
 AU Tari C; *Parulekar S J; Stark B C; Webster D A
 AN 1999-12957 BIOTECHDS
- L61 ANSWER 24 OF 134 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
 TI **Sequence** of archaeal Methanococcus jannaschii **alpha-**
amylase contains features of families 13 and 57 of glycosyl
 hydrolases: A trace of their common ancestor?
 SO FOLIA MICROBIOLOGICA, (FEB 1998) Vol. 43, No. 2, pp. 123-128.
 Publisher: FOLIA MICROBIOLOGICA, INST MICROBIOLOGY, VIDENSKA 1083, PRAGUE
 4, CZECH REPUBLIC 142 20.
 ISSN: 0015-5632.
 AU Janecek S (Reprint)
 AN 1998:208636 SCISEARCH
- L61 ANSWER 25 OF 134 HCAPLUS COPYRIGHT 2003 ACS on STN
 TI Extracellular .alpha.-amylase from Thermus filiformis Ork A2: purification
 and biochemical characterization
 SO Extremophiles (1998), 2(1), 23-32
 CODEN: EXTRFI; ISSN: 1431-0651
 AU Egas, Maria C. V.; da Costa, Milton S.; Cowan, Don A.; Pires, Euclides M.
 V.
 AN 1998:144647 HCAPLUS
 DN 128:241074

L61 ANSWER 26 OF 134 HCAPLUS COPYRIGHT 2003 ACS on STN
 TI Hyperthermostable extracellular alpha-amylase from pyrococcus furiosus
 SO Book of Abstracts, 216th ACS National Meeting, Boston, August 23-27
 (1998), BTEC-019 Publisher: American Chemical Society, Washington, D. C.
 CODEN: 66KYA2
 AU Savchenko, A.; Dong, G.; Vieille, C.; Zeikus, G. J.
 AN 1998:528470 HCAPLUS

L61 ANSWER 27 OF 134 MEDLINE on STN DUPLICATE 8
 TI **alpha-Amylase gene of thermophilic**
 Streptomyces sp. T01: nucleotide sequence, transcriptional and amino acid
 sequence analysis.
 SO FEMS MICROBIOLOGY LETTERS, (1998 Mar 1) 160 (1) 17-23.
 Journal code: 7705721. ISSN: 0378-1097.
 AU Mellouli L; Ghorbel R; Virolle M J; Bejar S
 AN 1998156111 MEDLINE

L61 ANSWER 28 OF 134 MEDLINE on STN DUPLICATE 9
 TI Isolation and analysis of genes for amylolytic enzymes of the
hyperthermophilic bacterium Thermotoga maritima.
 SO FEMS MICROBIOLOGY LETTERS, (1998 Jan 1) 158 (1) 9-15.
 Journal code: 7705721. ISSN: 0378-1097.
 AU Bibel M; Brettl C; Gosslar U; Kriegshauser G; Liebl W
 AN 1998115241 MEDLINE

L61 ANSWER 29 OF 134 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
 TI Hyperthermostable extracellular alpha-amylase from Pyrococcus furiosus;
thermophilic bacterium recombinant enzyme production and
 characterization (conference abstract)
 SO Abstr.Pap.Am.Chem.Soc.; (1998) 216 Meet. Pt.3, BTEC019
 CODEN: ACSRAL ISSN: 0065-7727
 216th ACS National Meeting, Boston, MA, USA, 23-27 August, 1998, 216
 Meet., Pt.3, 1998.
 AU Savchenko A; Dong G; Vieille C; Zeikus G J
 AN 1999-14174 BIOTECHDS

L61 ANSWER 30 OF 134 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
 TI Vector for secretion for use in lactic acid bacteria, and production of a
 protein by use of the vector;
 recombinant enzyme e.g. amylase, peptidase or protease production
 AN 1997-13446 BIOTECHDS
 PI JP 09234078 9 Sep 1997

L61 ANSWER 31 OF 134 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
 TI Termamyl-like alpha-amylase variants with improved properties;
 enzyme engineering and expression in Bacillus spp.
 AU Svensden A; Borchert T V; Bisgard-Frantzen H
 AN 1998-01800 BIOTECHDS
 PI WO 9741213 6 Nov 1997

L61 ANSWER 32 OF 134 HCAPLUS COPYRIGHT 2003 ACS on STN
 TI Cloning and expression of the **gene** encoding super-thermostable .
alpha.-amylase the hyperthermophilic
 Pyrococcus strain KOD-1, and characterization and use of the enzyme
 SO Jpn. Kokai Tokkyo Koho, 12 pp.
 CODEN: JKXXAF
 IN Imanaka, Tadayuki; Tachibana, Yoshinaga; Suzuki, Yuji; Kojima, Iwao;
 Utsura, Kensaku
 AN 1997:470120 HCAPLUS
 DN 127:77927

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 09173077	A2	19970708	JP 1996-191138	19960719

- L61 ANSWER 33 OF 134 MEDLINE on STN DUPLICATE 11
 TI Cloning, sequencing, characterization, and expression of an extracellular alpha-amylase from the **hyperthermophilic** archaeon *Pyrococcus furiosus* in *Escherichia coli* and *Bacillus subtilis*.
 SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Jun 27) 272 (26) 16335-42.
 Journal code: 2985121R. ISSN: 0021-9258.
 AU Jorgensen S; Vorgias C E; Antranikian G
 AN 97341170 MEDLINE
- L61 ANSWER 34 OF 134 MEDLINE on STN DUPLICATE 12
 TI Application of the extracellular **alpha-amylase gene** from *Streptococcus bovis* 148 to construction of a secretion vector for yogurt starter strains.
 SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1997 Nov) 63 (11) 4593-6.
 Journal code: 7605801. ISSN: 0099-2240.
 AU Satoh E; Ito Y; Sasaki Y; Sasaki T
 AN 1998027396 MEDLINE
- L61 ANSWER 35 OF 134 MEDLINE on STN DUPLICATE 13
 TI Cloning, sequencing, and expression of the **gene** encoding extracellular **alpha-amylase** from *Pyrococcus furiosus* and biochemical characterization of the recombinant enzyme.
 SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1997 Sep) 63 (9) 3569-76.
 Journal code: 7605801. ISSN: 0099-2240.
 AU Dong G; Vieille C; Savchenko A; Zeikus J G
 AN 97438520 MEDLINE
- L61 ANSWER 36 OF 134 MEDLINE on STN DUPLICATE 14
 TI Characterization of the gene encoding an extracellular laccase of *Myceliophthora thermophila* and analysis of the recombinant enzyme expressed in *Aspergillus oryzae*.
 SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1997 Aug) 63 (8) 3151-7.
 Journal code: 7605801. ISSN: 0099-2240.
 AU Berka R M; Schneider P; Golightly E J; Brown S H; Madden M; Brown K M; Halkier T; Mondorf K; Xu F
 AN 97394941 MEDLINE
- L61 ANSWER 37 OF 134 MEDLINE on STN DUPLICATE 15
 TI Properties and **gene** structure of the *Thermotoga maritima* **alpha-amylase** AmyA, a putative lipoprotein of a **hyperthermophilic** bacterium.
 SO JOURNAL OF BACTERIOLOGY, (1997 Feb) 179 (3) 941-8.
 Journal code: 2985120R. ISSN: 0021-9193.
 AU Liebl W; Stemplinger I; Ruile P
 AN 97158692 MEDLINE
- L61 ANSWER 38 OF 134 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN DUPLICATE 16
 TI Study of stability of recombinant plasmids during the continuous culture of *Bacillus stearothermophilus* NUB3621 in nonselective medium
 SO BIOTECHNOLOGY AND BIOENGINEERING, (5 MAR 1997) Vol. 53, No. 5, pp. 507-514.
 Publisher: JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012.
 ISSN: 0006-3592.
 AU Brigidi P (Reprint); GonzalezVara A; Rossi M; Matteuzzi D
 AN 97:193236 SCISEARCH
- L61 ANSWER 39 OF 134 LIFESCI COPYRIGHT 2003 CSA on STN DUPLICATE 17
 TI Study of stability of recombinant plasmids during the continuous culture of *Bacillus stearothermophilus* NUB3621 in nonselective medium
 SO BIOTECHNOL. BIOENG., (1997) vol. 53, no. 5, pp. 507-514.
 ISSN: 0006-3952.
 AU Brigidi, P.; Gonzalez-Vara, A.; Rossi, M.; Matteuzzi, D.

- AN 97:100335 LIFESCI
- L61 ANSWER 40 OF 134 MEDLINE on STN DUPLICATE 18
 TI A **gene** encoding for an **alpha-amylase** from
thermophilic *Bacillus* sp. strain TS-23 and its expression in
Escherichia coli.
 SO JOURNAL OF APPLIED MICROBIOLOGY, (1997 Mar) 82 (3) 325-34.
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gene from the **hyperthermophilic** archaeon *Pyrococcus* sp.
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 facilitates use of enzyme in industrial processes when cloned in
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Pyrococcus furiosus recombinant pullulanase, alpha-glucosidase,
 beta-glucosidase or protease production in *Escherichia coli* or

Bacillus subtilis

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Bacillus licheniformis;
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glucose-isomerase
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secretion of foreign extracellular and intracellular proteins in *Bacillus*
subtilis;
penicillinase, **alpha-amylase**, cellulase and
beta-galactosidase **gene** c loning, expression and protein
secretion using a plasmid pISA412 or p lasmid pISAts412 vector

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alpha-amylase gene and its high expression;
cloning and DNA sequence

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gene cloning, expression and protein secretion in e.g. *Bacillus*
subtilis using a plasmid pKTH1910, plasmid pKTH1975 or plasmid
pKTH1912 vector containing a signal peptide sequence

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Bacillus licheniformis gene cloning and expression in *Bacillus*
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 nucleotide **sequence** of the gene, processing of the enzyme, and
 comparison to other **alpha-amylases**;
 gene cloning in *Escherichia coli*; DNA sequence
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 starch saccharification using beta-amylase, pullulanase, isoamylase and recombinant **alpha-amylase**; *Bacillus stearothermophilus* **gene** cloning in *Bacillus subtilis*
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 Streptococcus pyogenes expression DNA sequence EU19035 isolation and characterization; plasmid pCB22 vector construction; **alpha-amylase gene** cloning in *Bacillus subtilis*
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Bacillus stearothermophilus **alpha-amylase** and *Clostridium thermocellum* cellulase **gene** cloning; application to silage starter culture strain improvement (conference abstract)
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alpha-amylase production of a recombinant *Escherichia*
coli strain;
Bacillus stearothermophilus recombinant enzyme preparation;
Vitreoscilla sp. hemoglobin gene cloning, vector plasmid pMK79
 construction, expression; aerobic growth in microaerophilic conditions
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COST IN U.S. DOLLARS

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TOTAL

FULL ESTIMATED COST

ENTRY

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205.39

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STN INTERNATIONAL LOGOFF AT 15:04:39 ON 19 SEP 2003

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	5674	alpha adj amylase\$1	USPAT; US-PGPUB	2003/09/19 14:54
2	L2	5392	hyperthermophil\$ or thermophil\$	USPAT; US-PGPUB	2003/09/19 14:54
3	L3	679	1 and 2	USPAT; US-PGPUB	2003/09/19 14:54
4	L4	1540	1 near8 (gene\$1 or sequence\$1)	USPAT; US-PGPUB	2003/09/19 14:55
5	L5	221	4 and 2	USPAT; US-PGPUB	2003/09/19 14:55
6	L6	26	4 same 2	USPAT; US-PGPUB	2003/09/19 14:55

PGPUB-DOCUMENT-NUMBER: 20030135885

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030135885 A1

TITLE: Self-processing plants and plant parts

PUBLICATION-DATE: July 17, 2003

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Chen, Wen	Cary	NC	US	
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Kinkema, Mark	Durham	NC	US	

APPL-NO: 10/ 228063

DATE FILED: August 27, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60315281 20010827 US

US-CL-CURRENT: 800/284, 435/200, 435/320.1, 435/419, 435/6, 435/69.1
, 536/23.2, 800/294

ABSTRACT:

The invention provides polynucleotides, preferably synthetic polynucleotides, which encode processing enzymes that are optimized for expression in plants. The polynucleotides encode mesophilic, thermophilic, or hyperthermophilic processing enzymes, which are activated under suitable activating conditions to act upon the desired substrate. Also provided are "self-processing" transgenic plants, and plant parts, e.g., grain, which express one or more of these enzymes and have an altered composition that facilitates plant and grain processing. Methods for making and using these plants, e.g., to produce food products having improved taste and to produce fermentable substrates for the production of ethanol and fermented beverages are also provided.

RELATED APPLICATIONS

[0001] This application claims priority to Application Serial No. 60/315,281, filed Aug. 27, 2001, which is herein incorporated by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX (22):

[0020] Moreover, the present invention encompasses a plant stably transformed with the vectors of the present invention. A plant stably transformed with a vector comprising an alpha.-amylase having an amino acid sequence of any of SEQ ID NO: 1, 10, 13, 14, 15, 16, 33, or 35, or encoded by a polynucleotide comprising any of SEQ ID NO: 2 or 9 is provided. Preferably, the .alpha.-amylase is hyperthermophilic.

Summary of Invention Paragraph - BSTX (36):

[0034] In a most preferred embodiment, a method of producing hypersweet corn comprising treating transformed corn or a part thereof, the genome of which is augmented with and expresses in the endosperm an expression cassette encoding an .alpha.-amylase, under conditions which activate the at least one enzyme so as to convert polysaccharides in the corn into sugar, yielding hypersweet corn is described. Preferably, the enzyme is hyperthermophilic and the hyperthermophilic .alpha.-amylase comprises the amino acid sequence of any of SEQ ID NO: 10, 13, 14, 15, 16, 33, or 35, or an enzymatically active fragment thereof having .alpha.-amylase activity.

Summary of Invention Paragraph - BSTX (38):

[0036] In another aspect of the invention, a method of preparing hydrolyzed starch product comprising treating a plant part comprising starch granules and at least one starch processing enzyme under conditions which activate the at least one enzyme thereby processing the starch granules to form an aqueous solution comprising a hydrolyzed starch product, wherein the plant part is obtained from a transformed plant, the genome of which is augmented with an expression cassette encoding at least one .alpha.-amylase; and collecting the aqueous solution comprising hydrolyzed starch product is described. Preferably, the alpha.-amylase is hyperthermophilic and more preferably, the hyperthermophilic .alpha.-amylase comprises the amino acid sequence of any of SEQ ID NO: 1, 10, 13, 14, 15, 16, 33, or 35, or an active fragment thereof having .alpha.-amylase activity. Preferably, the expression cassette comprises a polynucleotide selected from any of SEQ ID NO: 2, 9, 46, or 52, a complement thereof, or a polynucleotide that hybridizes to any of SEQ ID NO: 2, 9, 46, or 52 under low stringency hybridization conditions and encodes a polypeptide having .alpha.-amylase activity. Moreover, the invention further provides for the genome of the transformed plant further comprising a polynucleotide encoding a non-thermophilic starch-processing enzyme. Alternatively, the plant part may be treated with a non-hyperthermophilic starch-processing enzyme.

Detail Description Paragraph - DETX (33):

[0108] In another embodiment of the invention, the polynucleotide encodes a hyperthermophilic processing enzyme that is operably linked to a chloroplast (amyloplast) transit peptide (CTP) and a starch binding domain, e.g., from the waxy gene. An exemplary polynucleotide in this embodiment encodes SEQ ID NO:10 .alpha.-amylase linked to the starch binding domain from waxy). Other exemplary polynucleotides encode a hyperthermophilic processing enzyme linked to a signal sequence that targets the enzyme to the endoplasmic reticulum and

secretion to the apoplast (exemplified by a polynucleotide encoding SEQ ID NO:13, 27, or 30, which comprises the N-terminal sequence from maize .gamma.-zein operably linked to .alpha.-amylase, .alpha.-glucosidase, glucose isomerase, respectively), a hyperthermophilic processing enzyme linked to a signal sequence which retains the enzyme in the endoplasmic reticulum (exemplified by a polynucleotide encoding SEQ ID NO:14, 26, 28, 29, 33, 34, 35, or 36, which comprises the N-terminal sequence from maize .gamma.-zein operably linked to the hyperthermophilic enzyme, which is operably linked to SEKDEL, wherein the enzyme is .alpha.-amylase, malA .alpha.-glucosidase, T. maritima glucose isomerase, T. neapolitana glucose isomerase), a hyperthermophilic processing enzyme linked to an N-terminal sequence that targets the enzyme to the amyloplast (exemplified by a polynucleotide encoding SEQ ID NO:15, which comprises the N-terminal amyloplast targeting sequence from waxy operably linked to .alpha.-amylase), a hyperthermophilic fusion polypeptide which targets the enzyme to starch granules (exemplified by a polynucleotide encoding SEQ ID NO:16, which comprises the N-terminal amyloplast targeting sequence from waxy operably linked to an .alpha.-amylase/waxy fusion polypeptide comprising the waxy starch binding domain), a hyperthermophilic processing enzyme linked to an ER retention signal (exemplified by a polynucleotide encoding SEQ ID NO:38 and 39). Moreover, a hyperthermophilic processing enzyme may be linked to a raw-starch binding site having the amino acid sequence (SEQ ID NO:53), wherein the polynucleotide encoding the processing enzyme is linked to the maize-optimized nucleic acid sequence (SEQ ID NO:54) encoding this binding site.

Detail Description Paragraph - DETX (40):

[0115] A signal sequence such as the maize .gamma.-zein N-terminal signal sequence for targeting to the endoplasmic reticulum and secretion into the apoplast may be operably linked to a polynucleotide encoding a hyperthermophilic processing enzyme in accordance with the present invention (Torrent et al., 1997). For example, SEQ ID NOS: 13, 27, and 30 provides for a polynucleotide encoding a hyperthermophilic enzyme operably linked to the N-terminal sequence from maize .gamma.-zein protein. Another signal sequence is the amino acid sequence SEKDEL for retaining polypeptides in the endoplasmic reticulum (Munro and Pelham, 1987). For example, a polynucleotide encoding SEQ ID NOS:14, 26, 28, 29, 33, 34, 35, or 36, which comprises the N-terminal sequence from maize .gamma.-zein operably linked to a processing enzyme which is operably linked to SEKDEL. A polypeptide may also be targeted to the amyloplast by fusion to the waxy amyloplast targeting peptide (Klosgen et al., 1986) or to a starch granule. For example, the polynucleotide encoding a hyperthermophilic processing enzyme may be operably linked to a chloroplast (amyloplast) transit peptide (CTP) and a starch binding domain, e.g., from the waxy gene. SEQ ID NO:10 exemplifies .alpha.-amylase linked to the starch binding domain from waxy. SEQ ID NO:15 exemplifies the N-terminal sequence amyloplast targeting sequence from waxy operably linked to .alpha.-amylase. Moreover, the polynucleotide encoding the processing enzyme may be fused to target starch granules using the waxy starch binding domain. For example, SEQ ID NO:16 exemplifies a fusion polypeptide comprising the N-terminal amyloplast targeting sequence from waxy operably linked to an .alpha.-amylase/waxy fusion polypeptide comprising the waxy starch binding domain.

Detail Description Paragraph - DETX (118):

[0191] The invention provides a method to produce dextrans and altered starches from a plant, or a product from a plant, that has been transformed with a processing enzyme which hydrolyses certain covalent bonds of a polysaccharide to form a polysaccharide derivative. In one embodiment, a plant, or a product of the plant such as a fruit or grain, or flour made from the grain that expresses the enzyme is placed under conditions sufficient to activate the enzyme and convert polysaccharides contained within the plant into polysaccharides of reduced molecular weight. Preferably, the enzyme is fused to a signal sequence that targets the enzyme to a starch granule, an amyloplast, the apoplast or to the endoplasmic reticulum as disclosed herein. The dextrin or derivative starch produced may then be isolated or recovered from the plant or the product of the plant. In another embodiment, a processing enzyme able to convert polysaccharides into dextrans or altered starches is placed under the control of an inducible promoter according to methods known in the art and disclosed herein. The plant is grown to a desired stage and the promoter is induced causing expression of the enzyme and conversion of the polysaccharides, within the plant or product of the plant, to dextrans or altered starches. Preferably the enzyme is alpha-amylase, pullulanase, iso or neo-pullulanase and is operably linked to a signal sequence that targets the enzyme to a starch granule, an amyloplast, the apoplast or to the endoplasmic reticulum. In one embodiment, the enzyme is targeted to the apoplast or to the endoreticulum. In yet another embodiment, a transformed plant is produced that expresses an enzyme able to convert starch into dextrans or altered starches. The enzyme is fused to a signal sequence that targets the enzyme to a starch granule within the plant. Starch is then isolated from the transformed plant that contains the enzyme expressed by the transformed plant. The enzyme contained in the isolated starch may then be activated under conditions sufficient for activation to convert the starch into dextrans or altered starches. Examples of hyperthermophilic enzymes, for example, able to convert starch to hydrolyzed starch products are provided herein. The methods may be used with any plant which produces a polysaccharide and that can express an enzyme able to convert a polysaccharide into sugar.

Detail Description Paragraph - DETX (123):

[0196] The invention also provides for the production of improved corn varieties (and varieties of other crops) that have normal levels of starch accumulation, and accumulate sufficient levels of amylolytic enzyme(s) in their endosperm, or starch accumulating organ, such that upon activation of the enzyme contained therein, such as by boiling or heating the plant or a part thereof in the case of a hyperthermophilic enzyme, the enzyme(s) is activated and facilitates the rapid conversion of the starch into simple sugars. These simple sugars (primarily glucose) will provide sweetness to the treated corn. The resulting corn plant is an improved variety for dual use as a grain producing hybrid and as sweet corn. Thus, the invention provides a method to produce hyper-sweet corn, comprising treating transformed corn or a part thereof, the genome of which is augmented with and expresses in endosperm an expression cassette comprising a promoter operably linked to a first polynucleotide encoding at least one amylolytic enzyme, conditions which activate the at least one enzyme so as to convert polysaccharides in the corn into sugar, yielding hypersweet corn. The promoter may be a constitutive promoter, a seed-specific promoter, or an endosperm-specific promoter which is

linked to a polynucleotide sequence which encodes a processing enzyme such as .alpha.-amylase, e.g., one comprising SEQ ID NO:13, 14, or 16. Preferably, the enzyme is hyperthermophilic. In one embodiment, the expression cassette further comprises a second polynucleotide which encodes a signal sequence operably linked to the enzyme encoded by the first polynucleotide. Exemplary signal sequences in this embodiment of the invention direct the enzyme to apoplast, the endoplasmic reticulum, a starch granule, or to an amyloplast. The corn plant is grown such that the ears with kernels are formed and then the promoter is induced to cause the enzyme to be expressed and convert polysaccharide contained within the plant into sugar.

Detail Description Paragraph - DETX (162):

[0232] The 797GL3 .alpha.-amylase, having the amino acid sequence SEQ ID NO:1, was selected for its hyperthermophilic activity. This enzyme's nucleic acid sequence was deduced and maize-optimized as represented in SEQ ID NO:2. Similarly, the 6gp3 pullulanase was selected having the amino acid sequence set forth in SEQ ID NO:3. The nucleic acid sequence for the 6gp3 pullulanase was deduced and maize-optimized as represented in SEQ ID NO:4.

Claims Text - CLTX (136):

135. The method of claim 134, wherein the hyperthermophilic .alpha.-amylase comprises the amino acid sequence of any of SEQ ID NO:10, 13, 14, 15, 16, 33, or 35, or an enzymatically active fragment thereof having .alpha.-amylase activity.

Claims Text - CLTX (153):

152. The method of claim 151, wherein the hyperthermophilic .alpha.-amylase comprises the amino acid sequence of any of SEQ ID NO: 1, 10, 13, 14, 15, 16, 33, or 35, or an active fragment thereof having .alpha.-amylase activity.

PGPUB-DOCUMENT-NUMBER: 20030125534

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030125534 A1

TITLE: Enzymes having alpha amylase activity and methods of
use thereof

PUBLICATION-DATE: July 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Callen, Walter	San Diego	CA	US	
Richardson, Toby	San Diego	CA	US	
Frey, Gerhard	San Diego	CA	US	
Short, Jay M.	Rancho Santa Fe	CA	US	
Mathur, Eric J.	Carlsbad	CA	US	
Gray, Kevin A.	San Diego	CA	US	
Kerovuo, Janne E.	San Diego	CA	US	
Slupska, Malgorzata	San Diego	CA	US	

APPL-NO: 10/ 081872

DATE FILED: February 21, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60270495 20010221 US

non-provisional-of-provisional 60270496 20010221 US

non-provisional-of-provisional 60291122 20010514 US

US-CL-CURRENT: 536/23.1

ABSTRACT:

The invention relates to alpha amylases and to polynucleotides encoding the alpha amylases. In addition methods of designing new alpha amylases and methods of use thereof are also provided. The alpha amylases have increased activity and stability at acidic, neutral and alkaline pH and increased temperature.

RELATED APPLICATION DATA

[0001] This application claims priority of U.S. Provisional Application No. 60/270,495, filed Feb. 21, 2001, now pending; U.S. Provisional Application No. 60/270,496, filed Feb. 21, 2001, now pending; and U.S. Provisional Application No. 60/291,122, filed May 14, 2001, now pending, all of which are herein incorporated by reference in their entirety.

----- KWIC -----

Detail Description Paragraph - DETX (516):

[0547] An Initial bioinformatic analysis was made with the known hyperthermophilic .alpha.-amylase sequences. FIG. 14a shows an alignment of the sequences some of which have been deposited at the NCBI database. This analysis revealed the potential for designing degenerate primers to PCR the entire gene minus its signal sequence (see FIG. 14a), yielding potentially novel full-length alpha amylases from a library.

PGPUB-DOCUMENT-NUMBER: 20020106779

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020106779 A1

TITLE: Thermostable peptidase

PUBLICATION-DATE: August 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cheng, Timothy C.	Pasadena	CA	US	
Ramakrishnan, Vij	Pasadena	CA	US	
Chan, Sunney I.	Pasadena	CA	US	

APPL-NO: 09/ 969125

DATE FILED: September 24, 2001

RELATED-US-APPL-DATA:

child 09969125 A1 20010924

parent division-of 09333768 19990615 US GRANTED

parent-patent 6294367 US

non-provisional-of-provisional 60089398 19980615 US

US-CL-CURRENT: 435/226, 435/252.3 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

Thermostable peptidase enzyme derived from archaeon from the genus *Pyrococcus* is disclosed. The enzyme is produced from native or recombinant host cells and can be utilized in the biotechnology industry as a useful enzyme in sequencing reactions.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from Provisional Application Serial No. 60/089,398, filed Jun. 15, 1998, which is incorporated herein by reference in its entirety and to which application a priority claim is made under 35 U.S.C. .sctn.119(e).

----- KWIC -----

Summary of Invention Paragraph - BSTX (5):

[0005] Most of the proteins isolated from these hyperthermophiles exhibit a temperature optimum of at least 80-100.degree. C. or above (Adams et al., Bio/Technology 13, 662-668 (1995); Adams et al., Trends Biotechnol 16, 329-332 (1998)). Accordingly, there is much interest in exploiting these proteins for biotechnological applications, as they are able to perform biochemical reactions under harsh conditions, such as in the presence of high-temperatures, organic solvents, and denaturants (Adams et al., supra.) P. furiosus has been the source of many of these biotechnologically important proteins, including DNA polymerase (Lundberg et al., Gene 108, 1-6 (1991)), .alpha.-amylase (Laderman et al., J. Biol. Chem. 268, 24394-24401 (1993)), and proteases (Voorhorst et al., J. Biol. Chem. 271, 20426-20431 (1996); Harwood et al., J. Bacteriol. 179, 3613-3618 (1997)).

US-PAT-NO: 6426211

DOCUMENT-IDENTIFIER: US 6426211 B1

TITLE: Xylanase derived from a Bacillus species, expression
vectors for such xylanase and other proteins, host
organisms therefor and use thereof

DATE-ISSUED: July 30, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
de Buyt; Eric	Linkebeek	N/A	N/A	BE
Lahaye; Andree	Brussels	N/A	N/A	BE
Ledoux; Pierre	Brussels	N/A	N/A	BE
Amory; Antoine	Rixensart	N/A	N/A	BE
Detroz; Rene	Ohain	N/A	N/A	BE
Andre; Christophe	Greze-Doiceau	N/A	N/A	BE
Vetter; Roman	Burgdorf	N/A	N/A	DE

APPL-NO: 09/ 073055

DATE FILED: May 5, 1998

PARENT-CASE:

This application is a divisional of 08/275,526 Jul. 15, 1994 now U.S. Pat.
No. 6,180,382, Jan. 30, 2001.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9314780	July 15, 1993

US-CL-CURRENT: 435/278, 435/183, 435/200, 435/252.3, 435/262, 435/267
, 435/274, 435/277, 435/320.1, 435/69.1

ABSTRACT:

A purified xylanase derived from B. Pumilus PRL B12 is disclosed. This xylanase is efficient for use in the biobleaching of wood pulp, permitting a strong reduction in the quantity of chlorine used and AOX compounds produced in classical and ECF wood pulp bleaching sequences as well as the quantity of ozone used in TCF sequences. The gene coding for the xylanase was isolated and purified and used to construct an expression vector therefor. A recombinant host strain of B. licheniformis is also disclosed which is efficient for expressing heterologous enzymes, including the xylanase when transformed by the expression vector.

16 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

----- KWIC -----

Detailed Description Text - DETX (68):

Other expression vectors provided herein include the nucleotide sequence that codes for the pullulanase of *Bacillus deramificans* T 89.117D (pUBDEBRA1), or the nucleotide sequence that codes for the .alpha.-amylase of B. licheniformis ATCC 9789 (pL7TAKA), or the nucleotide sequence that codes for the alkaline protease of *B. licheniformis* SE2 (pL11), or the subtilisin (alkaline protease) of *Bacillus subtilis* 168 (pKAC1 and pL7SBT). The expression hosts of the present invention are strains of the genus *Bacillus* which are compatible with the expression vector for the protein desired to be expressed thereby. Preferably, these strains are aerobic. It is further preferred that these strains not be thermophilic. Such strains include *B. subtilis*, *B. pumilus*, and *B. licheniformis*, *B. alkalophilus*, *B. lentus* and *B. amyloliquefaciens*. Preferably, the alkaline protease gene(s) thereof has (have) been deleted from these expression hosts.

US-PAT-NO: 6423523

DOCUMENT-IDENTIFIER: US 6423523 B1

TITLE: Xylanase derived from a bacillus species, expression vectors for such xylanase and other proteins, host organisms therefor and use thereof

DATE-ISSUED: July 23, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
de Buyl; Eric	Linkebeek	N/A	N/A	BE
Lahaye; Andree	Brussels	N/A	N/A	BE
Ledoux; Pierre	Brussels	N/A	N/A	BE
Amory; Antoine	Rixensart	N/A	N/A	BE
Detroz; Rene	Ohain	N/A	N/A	BE
Andre; Christophe	Grez-Doiceau	N/A	N/A	BE
Vetter; Roman	Burgdorf	N/A	N/A	DE

APPL-NO: 09/ 076677

DATE FILED: May 12, 1998

PARENT-CASE:

This application is a divisional of Ser. No. 08/275,526 filed Jul. 15, 1994 now U.S. Pat. No. 6,180,382, Jan. 30, 2001.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9314780	July 15, 1993

US-CL-CURRENT: 435/200, 435/183 , 435/194 , 435/252.3 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

A purified xylanase derived from *B. Pumilus* PRL B12 is disclosed. This xylanase is efficient for use in the biobleaching of wood pulp, permitting a strong reduction in the quantity of chlorine used and AOX compounds produced in classical and ECF wood pulp bleaching sequences as well as the quantity of ozone used in TCF sequences. The gene coding for the xylanase was isolated and purified and used to construct an expression vector therefor. A recombinant host strain of *B. licheniformis* is also disclosed which is efficient for expressing heterologous enzymes, including the xylanase when transformed by the expression vector.

25 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

----- KWIC -----

Detailed Description Text - DETX (68):

Other expression vectors provided herein include the nucleotide sequence that codes for the pullulanase of *Bacillus deramificans* T 89.117D (pUBDEBRA1), or the nucleotide sequence that codes for the .alpha.-amylase of B. licheniformis ATCC 9789 (pL7TAKA), or the nucleotide sequence that codes for the alkaline protease of *B. licheniformis* SE2 (pL11), or the subtilisin (alkaline protease) of *Bacillus subtilis* 168 (pKAC1 and pL7SBT). The expression hosts of the present invention are strains of the genus *Bacillus* which are compatible with the expression vector for the protein desired to be expressed thereby. Preferably, these strains are aerobic. It is further preferred that these strains not be thermophilic. Such strains include *B. subtilis*, *B. pumilus*, and *B. licheniformis*, *B. alkalophilus*, *B. lentus* and *B. amyloliquefaciens*. Preferably, the alkaline protease gene(s) thereof has (have) been deleted from these expression hosts.

US-PAT-NO: 6329187

DOCUMENT-IDENTIFIER: US 6329187 B1

TITLE: Endoglucanases

DATE-ISSUED: December 11, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lam; David E.	Harbor City	CA	N/A	N/A
Mathur; Eric J.	Carlsbad	CA	N/A	N/A

APPL-NO: 09/ 430669

DATE FILED: October 28, 1999

PARENT-CASE:

This application is a divisional of application Ser. No. 09/066,544 filed on Apr. 24, 1998, now U.S. Pat. No. 6,001,984, which is a continuation application of U.S. application Ser. No. 08/651,572, filed May 22, 1996, now issued as U.S. Pat. No. 5,789,228, the entire contents of which are hereby incorporated by reference herein.

US-CL-CURRENT: 435/209, 435/183 , 435/200 , 435/220 , 536/23.2

ABSTRACT:

A purified thermostable enzyme is derived from the archaeal bacterium AEPII1a. The enzyme has a molecular weight of about 60.9 kilodaltons and has cellulase activity. The enzyme can be produced from native or recombinant host cells and can be used to aid in the digestion of cellulose where desired.

8 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

----- KWIC -----

Other Reference Publication - OREF (24):

Tachibana Yoshihisa et al., "Cloning and Expression of the **Alpha-Amylase Gene from the Hyperthermophilic** Archaeon *Pyrococcus* SP. KOD1, and Characterization of the Enzyme", *Journal of Fermentation and Bioengineering*, vol. 82, No. 3, 1996 (pp. 224-232).

US-PAT-NO: 6300115

DOCUMENT-IDENTIFIER: US 6300115 B1

TITLE: Pullulanase expression constructs containing
.alpha.-amylase promoter and leader sequences

DATE-ISSUED: October 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Teague; W. Martin	Rockford	IL	N/A	N/A
Brumm; Phillip J.	Rockford	IL	N/A	N/A
Allen; Larry N.	Northfield	IL	N/A	N/A
Brikun; Igor A.	Forest Park	IL	N/A	N/A

APPL-NO: 09/ 313677

DATE FILED: May 18, 1999

PARENT-CASE:

Priority is claimed to provisional patent application Ser. No. 60/122,065, filed May 18, 1998, and incorporated herein by reference.

US-CL-CURRENT: 435/210, 435/252.31 , 435/254.11 , 435/320.1 , 435/325
, 435/419 , 536/23.1 , 536/23.2 , 536/23.4 , 536/24.1

ABSTRACT:

Disclosed herein are DNA expression constructs containing an .alpha.-amylase promoter sequence derived from *Bacillus stearothermophilus*, an .alpha.-amylase leader sequence derived from *Bacillus stearothermophilus*, and a DNA sequence encoding a pullulanase derived from *Bacillus naganoensis*. Microbial hosts transformed to contain the expression constructs secret function pullulanases. Also disclosed is a process for making recombinant pullulanases utilizing the expression constructs and a recombinant pullulanase which can be produced in *Bacillus subtilis*.

25 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

----- KWIC -----

Other Reference Publication - OREF (23):

Gray et al., Structural **Genes Encoding the Thermophilic .alpha.-Amylases** of
Bacillus stearothermophilus and Bacillus licheniformis, Journal of Bacteriology
(May 1986), 166:635-643.

US-PAT-NO: 6294367

DOCUMENT-IDENTIFIER: US 6294367 B1

TITLE: Thermostable peptidase

DATE-ISSUED: September 25, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cheng; Timothy C.	Pasadena	CA	N/A	N/A
Ramakrishnan; Vij	Pasadena	CA	N/A	N/A
Chan; Sunney I.	Pasadena	CA	N/A	N/A

APPL-NO: 09/ 333768

DATE FILED: June 15, 1999

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from Provisional Application Ser. No. 60/089,398, filed Jun. 15, 1998, which is incorporated herein by reference in its entirety and to which application a priority claim is made under 35 U.S.C. .sctn.119(e).

US-CL-CURRENT: 435/212, 435/252.3 , 435/320.1 , 435/325 , 435/455 , 435/6 , 435/69.1 , 536/23.2

ABSTRACT:

Thermostable peptidase enzyme derived from archaeon from the genus *Pyrococcus* is disclosed. The enzyme is produced from native or recombinant host cells and can be utilized in the biotechnology industry as a useful enzyme in sequencing reactions.

19 Claims, 18 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

----- KWIC -----

Brief Summary Text - BSTX (5):

Most of the proteins isolated from these hyperthermophiles exhibit a temperature optimum of at least 80-100.degree. C. or above (Adams et al.,

Bio/Technology 13, 662-668 (1995); Adams et al., Trends Biotechnol 16, 329-332 (1998)). Accordingly, there is much interest in exploiting these proteins for biotechnological applications, as they are able to perform biochemical reactions under harsh conditions, such as in the presence of high-temperatures, organic solvents, and denaturants (Adams et al., supra.) *P. furiosus* has been the source of many of these biotechnologically important proteins, including DNA polymerase (Lundberg et al., Gene 108, 1-6 (1991)), **.alpha.-amylase** (Laderman et al., J. Biol. Chem. 268, 24394-24401 (1993)), and proteases (Voorhorst et al., J. Biol. Chem. 271, 20426-20431 (1996); Harwood et al., J. Bacteriol. 179, 3613-3618 (1997)).

US-PAT-NO: 6218164

DOCUMENT-IDENTIFIER: US 6218164 B1

TITLE: Thermopallium bacteria and enzymes obtainable therefrom

DATE-ISSUED: April 17, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Jones; Brian E.	Leidschendam	N/A	N/A	NL
Herweijer; Margareta A.	The Hague	N/A	N/A	NL
Danson; Michael J.	Saltford	N/A	N/A	GB
Hough; David W.	Bath	N/A	N/A	GB
Thompson; Carl R.	Bath	N/A	N/A	GB

APPL-NO: 09/ 029937

DATE FILED: June 2, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	95202477	September 13, 1995

PCT-DATA:

APPL-NO: PCT/EP96/03896
DATE-FILED: September 3, 1996
PUB-NO: WO97/10342
PUB-DATE: Mar 20, 1997
371-DATE: Jun 2, 1998
102(E)-DATE: Jun 2, 1998

US-CL-CURRENT: 435/210, 435/201 , 435/277 , 435/278 , 435/98 , 510/300
, 510/305

ABSTRACT:

The present invention provides thermophilic alkaliphilic bacteria designated *Thermopallium natronophilum* and thermophilic alkaliphilic polypeptides obtainable therefrom. It also provides compositions, particularly detergent compositions comprising the polypeptides.

10 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Other Reference Publication - OREF (2):

Lee, S.-P., et al., Applied and Environmental Microbiology, vol. 60, Cloning of the aapT gene and characterization of its product, alpha-amylase-pullulanase (Aapt) from thermophilic and alkaliphilic Bacillus sp. strain XAL601, pp. 3764-3773, 1994.*

US-PAT-NO: 6187576

DOCUMENT-IDENTIFIER: US 6187576 B1

TITLE: .alpha.-amylase mutants

DATE-ISSUED: February 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svensden; Allan	Birker.o slashed.d	N/A	N/A	DK
Borchert; Torben Vedel	Jyllinge	N/A	N/A	DK
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd	N/A	N/A	DK

APPL-NO: 09/ 170670

DATE FILED: October 13, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

The application claims priority under 35 U.S.C 119 of Danish application 1172/97 filed Oct. 13, 1997, and of U.S. provisional application 60/063,306 filed Oct. 28, 1997, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1172/97	October 13, 1997

US-CL-CURRENT: 435/202, 435/183, 435/200, 510/226, 510/235, 510/320, 510/392

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like .alpha.-amylase, comprising mutations in two, three, four, five or six regions/positions. The variants have increased thermostability at acidic pH and/or at low Ca.sup.2+ concentrations (relative to the parent). The invention also relates to a DNA construct comprising a DNA sequence encoding an .alpha.-amylase variant of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an .alpha.-amylase variant of the invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an .alpha.-amylase variant of the invention, a manual or automatic dishwashing detergent composition comprising an .alpha.-amylase variant of the invention, a method for generating a variant of a parent Termamyl-like .alpha.-amylase, which variant exhibits increased thermostability at acidic pH and/or at low Ca.sup.2+ concentrations (relative

to the parent).

22 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

----- KWIC -----

Other Reference Publication - OREF (1):

Gray G.L. et al. Structural genes encoding the thermophilic alpha-amylases of *Bacillus stearothermophilus* and *B.licheniformis*. J.Bacteriol., May 1986, vol. 166(2):635-643.

US-PAT-NO: 6180382

DOCUMENT-IDENTIFIER: US 6180382 B1

TITLE: Xylanase derived from a bacillus species, expression
vectors for such xylanase and other proteins, host
organisms therefor and use thereof

DATE-ISSUED: January 30, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
De Buyl; Eric	B-1630 Linkebeek	N/A	N/A	BE
Lahaye; Andree	B-1020 Brussels	N/A	N/A	BE
Ledoux; Pierre	B-1200 Brussels	N/A	N/A	BE
Amory; Antoine	B-1330 Rixensart	N/A	N/A	BE
Detroz; Rene	B-1328 Ohain	N/A	N/A	BE
Andre; Christophe	B-1390 Grez-Doiceau	N/A	N/A	BE
Vetter; Roman	W-31303 Burgdorf	N/A	N/A	DE

APPL-NO: 08/ 275526

DATE FILED: July 15, 1994

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9314780	July 15, 1993

US-CL-CURRENT: 435/200, 435/183 , 435/201 , 435/220 , 435/222

ABSTRACT:

A purified xylanase derived from *B. Pumilus* PRL B12 is disclosed. This xylanase is efficient for use in the biobleaching of wood pulp, permitting a strong reduction in the quantity of chlorine used and AOX compounds produced in classical and ECF wood pulp bleaching sequences as well as the quantity of ozone used in TCF sequences. The gene coding for the xylanase was isolated and purified and used to construct an expression vector therefor. A recombinant host strain of *B. licheniformis* is also disclosed which is efficient for expressing heterologous enzymes, including the xylanase when transformed by the expression vector.

19 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

----- KWIC -----

Detailed Description Text - DETX (73):

Other expression vectors provided herein include the nucleotide sequence that codes for the pullulanase of *Bacillus deramificans* T 89.117D (pUBDEBRA1), or the nucleotide sequence that codes for the .alpha.-amylase of B. licheniformis ATCC 9789 (pL7TAKA), or the nucleotide sequence that codes for the alkaline protease of *B. licheniformis* SE2 (pLI1), or the subtilisin (alkaline protease) of *Bacillus subtilis* 168 (pKAC1 and pL7SBT). The expression hosts of the present invention are strains of the genus *Bacillus* which are compatible with the expression vector for the protein desired to be expressed thereby. Preferably, these strains are aerobic. It is further preferred that these strains not be thermophilic. Such strains include *B. subtilis*, *B. pumilus*, and *B. licheniformis*, *B. alkalophilus*, *B. lentus* and *B. amyloliquefaciens*. Preferably, the alkaline protease gene(s) thereof has (have) been deleted from these expression hosts.

US-PAT-NO: 6080568

DOCUMENT-IDENTIFIER: US 6080568 A

TITLE: Mutant .alpha.-amylase comprising modification at
residues corresponding to A210, H405 and/or T412 in
Bacillus licheniformis

DATE-ISSUED: June 27, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Day; Anthony G.	San Francisco	CA	N/A	N/A
Swanson; Barbara A.	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 914679

DATE FILED: August 19, 1997

US-CL-CURRENT: 435/202, 435/201, 435/203, 435/275, 435/440, 435/832
, 435/836, 510/320, 570/226, 570/235

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which one or more of residues corresponding to A210, H405 and T412 in Bacillus licheniformis are mutated. The disclosed .alpha.-amylase enzymes show altered or improved stability and/or activity profiles.

11 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

----- KWIC -----

Other Reference Publication - OREF (22):

Gray et al., "Structural **Genes Encoding the Thermophilic .alpha.-amylases** of Bacillus stearothermophilus and Bacillus licheniformis," J Bacteriol (1986) 166:635-643.

US-PAT-NO: 6022724

DOCUMENT-IDENTIFIER: US 6022724 A

TITLE: .alpha.-amylase mutants

DATE-ISSUED: February 8, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birkerød	N/A	N/A	DK
Bisg.ang.rd-Frantzen; Henrik	Lyngby	N/A	N/A	DK
Borchert; Torben	Copenhagen N	N/A	N/A	DK

APPL-NO: 08/ 683838

DATE FILED: July 18, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of Ser. No. 08/600,908 filed Feb. 13, 1996 which is a 371 of PCT/DK96/00057 filed Feb. 5, 1996, which are incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0128/95	February 3, 1995
DK	1192/95	October 23, 1995
DK	1256/95	November 10, 1995

US-CL-CURRENT: 435/202, 435/203 , 510/226 , 510/235 , 510/320 , 510/392

ABSTRACT:

The present invention relates to a method of constructing a variant of a parent Termamyl-like .alpha.-amylase, which variant has .alpha.-amylase activity and at least one altered property as compared to the parent .alpha.-amylase, comprises

i) analyzing the structure of the parent Termamyl-like .alpha.-amylase to identify at least one amino acid residue or at least one structural part of the Termamyl-like .alpha.-amylase structure, which amino acid residue or structural part is believed to be of relevance for altering the property of the parent Termamyl-like .alpha.-amylase (as evaluated on the basis of structural or functional considerations),

ii) constructing a Termamyl-like .alpha.-amylase variant, which as compared to the parent Termamyl-like .alpha.-amylase, has been modified in the amino

acid residue or structural part identified in i) so as to alter the property,
and, optionally,

iii) testing the resulting Termamyl-like α -amylase variant with
respect to the property in question.

5 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

----- KWIC -----

Other Reference Publication - OREF (5):

Gray et al., "Structural Genes Encoding The Thermophilic α -Amylases of
Bacillus Stearothermophilus And Bacillus Licheniformis", Journal of
Bacteriology, vol. 166, No. 2, May 1996, pp. 635-643.

US-PAT-NO: 5981243

DOCUMENT-IDENTIFIER: US 5981243 A

TITLE: Purified myceliophthora laccases and nucleic acids
encoding same

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Berka; Randy Michael	Davis	CA	95616	N/A
Brown; Stephen H.	Davis	CA	95616	N/A
Xu; Feng	Woodland	CA	95776	N/A
Schneider; Palle	DK-2750 Ballerup	N/A	N/A	DK
Oxenb.o slashed.II; Karen M.	DK-2920 Charlottenlund	N/A	N/A	DK
Aaslyng; Dorrit A.	Gartnerkrogen 69	N/A	N/A	DK

APPL-NO: 08/ 939218

DATE FILED: September 29, 1997

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 08/441,146 filed May 15, 1995, now abandoned, which a continuation-in-part of application Ser. No. 08/253,781 filed Jun. 3, 1994, now abandoned, the contents of which are fully incorporated herein by reference.

US-CL-CURRENT: 435/189, 536/23.2 , 8/401

ABSTRACT:

The present invention relates to isolated nucleic acid constructs containing a sequence encoding a Myceliophthora laccase, and the laccase proteins encoded thereby.

18 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

----- KWIC -----

Detailed Description Text - DETX (55):

The construction strategy for the laccase expression vector pRaMB5 is outlined in FIG. 3. The promoter directing transcription of the laccase gene is obtained from the A. oryzae .alpha.-amylase (TAKA-amylase) gene (Christensen et al., supra), as well as the TAKA-amylase terminator region. The plasmid is constructed first by modifying pMWR3 by inserting a small linker which contains an Apal site between the Swal and Nsil sites, creating a plasmid called pMWR3-SAN. Pful polymerase-directed PCR (Stratagene, La Jolla, Calif.) is used to amplify a short DNA segment encoding the 5'-portion of MtL, from the start codon to an internal PstI site (approximately 0.5 kb). The forward primer for this PCR reaction is designed to create an EcoRI site just upstream of the start codon. Next, the amplified fragment is digested with EcoRI and PstI[during this step, the EcoRI site is made blunt by treatment with dNTPs and DNA polymerase I(Klenow fragment)] and purified by agarose gel electrophoresis. The 3' portion of the M. thermophila coding region is excised from pRaMB2 as a 2kb PstI-Apal fragment(this segment also contains approximately 110 bp from the 3'-untranslated region). These two fragments are combined with Swal- and Apal-cleaved pMWR3-SAN in a three-part ligation reaction to generate the laccase expression vector pRaMB5.

US-PAT-NO: 5958739

DOCUMENT-IDENTIFIER: US 5958739 A

TITLE: Mutant .alpha.-amylase

DATE-ISSUED: September 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitchinson; Colin	Palo Alto	CA	N/A	N/A
Requadt; Carol	Palo Alto	CA	N/A	N/A
Ropp; Traci	Palo Alto	CA	N/A	N/A
Solheim; Leif P.	Palo Alto	CA	N/A	N/A
Ringer; Christopher	Palo Alto	CA	N/A	N/A
Day; Anthony	Palo Alto	CA	N/A	N/A

APPL-NO: 08/ 704706

DATE FILED: February 20, 1997

PCT-DATA:

APPL-NO: PCT/US96/09089

DATE-FILED: June 6, 1996

PUB-NO: WO96/39528

PUB-DATE: Dec 19, 1996

371-DATE: Feb 20, 1997

102(E)-DATE:Feb 20, 1997

US-CL-CURRENT: 435/99, 435/201 , 435/202 , 435/203 , 435/204 , 435/252.3
 , 435/252.31 , 435/254.11 , 435/320.1 , 435/325 , 435/410
 , 510/226 , 510/300 , 510/305 , 510/320 , 510/374 , 510/392
 , 536/23.2

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which one or more asparagine residues are substituted with a different amino acid or deleted. The disclosed .alpha.-amylase enzymes show altered or improved low pH starch hydrolysis performance, stability and activity profiles.

32 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

----- KWIC -----

Other Reference Publication - OREF (26):

Gray et al., "Structural Genes Encoding the Thermophilic .alpha.-amylases of Bacillus stearothermophilus and Bacillus licheniformis," J Bacteriol (1986) 166:635-643.

US-PAT-NO: 5849549

DOCUMENT-IDENTIFIER: US 5849549 A

TITLE: Oxidatively stable alpha-amylase

DATE-ISSUED: December 15, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP	CODE	COUNTRY
Barnett; Christopher C.	South San Francisco	CA	N/A	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A	N/A
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A	N/A
Power; Scott D.	San Bruno	CA	N/A	N/A	N/A
Requadt; Carol A.	Tiburon	CA	N/A	N/A	N/A

APPL-NO: 08/ 468698

DATE FILED: June 6, 1995

PARENT-CASE:

RELATED APPLICATION

This is a divisional of U.S. Ser. No. 08/194,664 filed Feb. 10, 1994, now pending, which is a continuation-in-part of U.S. Ser. No. 08/016,395 filed Feb. 11, 1993, abandoned.

US-CL-CURRENT: 435/99, 435/202 , 536/23.2

ABSTRACT:

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such mutant alpha-amylases have altered oxidative stability and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

2 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

----- KWIC -----

Other Reference Publication - OREF (5):

Gray, et al., "Structural Genes Encoding the Thermophilic .alpha.-amylases
of *Bacillus sterothermophilus* and *Bacillus licheniformis*" J. Bact.
166(2):635-643 (May 1986).

US-PAT-NO: 5840851

DOCUMENT-IDENTIFIER: US 5840851 A

TITLE: Purification of hemoglobin

DATE-ISSUED: November 24, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Plomer; J. Jeffrey	Broomfield	CO	80020	N/A
Ryland; James R.	Louisville	CO	80027	N/A
Matthews; Maura-Ann H.	Boulder	CO	80304	N/A
Traylor; David W.	Wheat Ridge	CO	80033	N/A
Milne; Erin E.	Broomfield	CO	80020	N/A
Durfee; Steven L.	Denver	CO	80207	N/A
Mathews; Antony J.	Louisville	CO	80027	N/A
Neway; Justinian O.	Longmont	CO	80503	N/A

APPL-NO: 08/ 438511

DATE FILED: May 10, 1995

PARENT-CASE:

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a continuation-in-part of application Ser. No. 08/339,304, filed Nov. 14, 1994, now abandoned which is a continuation-in-part of application Ser. No. Ser. No. 08/097,273, filed Jul. 23, 1993, now abandoned, both incorporated herein by reference in their entirety.

US-CL-CURRENT: 530/385, 530/412 , 530/413 , 530/416

ABSTRACT:

The present invention generally relates to methods for purifying hemoglobin solutions and to hemoglobin solutions obtained by the methods. In one aspect, such methods include removing contaminants in crude hemoglobin-containing lysates with heat treatment. In a further aspect, the present invention provides methods for producing substantially purified hemoglobin solutions using immobilized metal affinity chromatography, optionally following by anion exchange chromatography.

51 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

----- KWIC -----

Other Reference Publication - OREF (34):

Tsukagoshi, N. et al/Cloning and Expression of a Thermophilic
.alpha.-Amylase Gene from Bacillus Stearothermophilus in Escherichia Coli/Mol.
Gen Genet/(1984)/193: 58-63.

US-PAT-NO: 5824532

DOCUMENT-IDENTIFIER: US 5824532 A

TITLE: Oxidativley stable alpha-amylase

DATE-ISSUED: October 20, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barnett; Christopher C.	South San Francisco	CA	N/A	N/A
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Power; Scott D.	San Bruno	CA	N/A	N/A
Requadt; Carol A.	Tiburon	CA	N/A	N/A

APPL-NO: 08/ 468220

DATE FILED: June 6, 1995

PARENT-CASE:

RELATED APPLICATIONS

This is a divisional of U.S. Ser. No. 08/194,664 filed Feb. 10, 1994, now pending which is a continuation-in-part of U.S. Ser. No. 08/016,395 filed Feb. 11, 1993 now abandoned.

US-CL-CURRENT: 435/202, 435/201 , 435/203 , 435/204 , 435/252.3 , 435/252.31 , 435/320.1 , 435/71.2 , 536/23.2 , 536/23.7

ABSTRACT:

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such mutant alpha-amylases have altered oxidative stability and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

11 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

----- KWIC -----

Other Reference Publication - OREF (2):

G. Gray et al., "Structural Gene Encoding the Thermophilic .alpha.-Amylases
of *Bacillus stearothermophilus* and *Bacillus licheniformis*", J. Bact. 166(2)
635-643, (May 1986).

US-PAT-NO: 5756714

DOCUMENT-IDENTIFIER: US 5756714 A

TITLE: Method for liquefying starch

DATE-ISSUED: May 26, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Antrim; Richard L.	Solon	IA	N/A	N/A
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A

APPL-NO: 08/ 411038

DATE FILED: March 27, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Ser. No. 08/401,325 filed Mar. 9, 1995, now abandoned and which is incorporated herein by reference in its entirety.

US-CL-CURRENT: 536/102, 435/202, 435/203, 435/204, 435/205, 435/96, 435/99

ABSTRACT:

According to the invention a method is provided for liquefying starch comprising the steps of treating the starch prior to or simultaneously with liquefying the starch to inactivate and/or remove the enzyme inhibiting composition present in the starch and form treated starch; adding .alpha.-amylase to the treated starch; and reacting the treated starch for a time and at a temperature effective to liquefy the treated starch. Effective means to treat the starch include the addition of a phytate degrading enzyme and heat treatment, optionally followed by filtration or centrifugation, of granular starch or a starch solution.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Other Reference Publication - OREF (8):

Gray, et al., "Structural Genes Encoding the Thermophilic .alpha.-Amylases
of *Bacillus sterothermophilus* and *Bacillus licheniformis*" J. of Bacteriology
166(2):635-643 (May 1986).

US-PAT-NO: 5707841

DOCUMENT-IDENTIFIER: US 5707841 A

TITLE: Process of producing highly transformable bacterial cells and cells produced thereby

DATE-ISSUED: January 13, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Greener; Alan L.	San Diego	CA	N/A	N/A

APPL-NO: 08/ 637003

DATE FILED: April 18, 1996

PARENT-CASE:

This is a continuation of application Ser. No. 08,151,577, filed Nov. 12, 1993, now U.S. Pat. No. 5,512,468.

US-CL-CURRENT: 435/488, 435/252.33 , 435/252.8

ABSTRACT:

The invention provided herein includes gram negative bacteria cells containing a gene encoding an enzyme with carbohydrate degrading activity that had been rendered competent to transformation. Carbohydrate degrading enzymes of interest for use in the invention include alpha-amylase. The competent cells of the subject invention may be frozen so as to provide for prolonged storage. Other aspects of the invention include methods for rendering gram negative bacterial cells, such as E. coli cells competent to transformation. These methods involve the step of transferring a gene encoding an enzyme with carbohydrate degrading activity into E. coli cells and subsequently rendering the cells competent using any of a variety of competency inducing procedures.

13 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX (14):

The term carbohydrate-degrading enzyme as used herein refers to enzymes capable of hydrolyzing at least one type of linkage present between the constituent monosaccharide units of a carbohydrate molecule. The term "starch-degrading enzyme" as used herein refers to enzymes capable of

hydrolyzing at least one type of linkage present between the constituent monosaccharide units of a starch molecule. The term "alpha-amylase" as used herein refers to enzymes capable of catalyzing hydrolysis of the .alpha.-1.fwdarw.4 glucosidic linkages of polysaccharides containing such glucosidic linkages such as starch or glycogen. Preferred carbohydrate degrading enzymes are starch degrading enzymes. Preferred starch degrading enzymes are alpha-amylases. Particularly preferred starch degrading enzymes for use in the invention are alpha-amylases isolated from thermophilic bacteria, especially the alpha-amylase gene from a recently isolated uncharacterized thermophilic bacterium. The polynucleotide sequence encoding this alpha-amylase from the uncharacterized thermophilic bacterium can be found on the FAMY plasmid present in the E. coli strains having the ATCC accession numbers 69480, 69481, and 69482.

Brief Summary Text - BSTX (18):

The introduction of a genetic construction for the expression of a carbohydrate degrading enzyme into E. coli serves to increase the transformation efficiency of compositions of E. coli cells rendered competent for a wide variety of E. coli strains. The genotype of an E. coli cell strain containing a genetic construction for the expression of a carbohydrate degrading enzyme may be selected so as to be particularly useful for a given genetic engineering experiment. For example, cloning vectors that are screenable because of LacZ.alpha. fragment complementation may contain a particular mutation within the LacZ gene. Similarly, the cell may contain various other deletions or mutations in order to provide for complementation by the transforming DNA. The host cell may either possess or lack a restriction-modification system in order to expedite cloning. The host cells may also lack one or more recombination systems, e.g., RecA, RecBC. Preferred E. coli strains for use in the invention are cells that contain a mutation in the deoR gene, as described in Hanahan, U.S. Pat. No. 4,851,348. Particularly preferred strains of E. coli for use in the invention are the XL1-Blue.TM. strain (Stratagene, La Jolla, Calif.), the XL1-Blue MR strain, and the SURE.TM. strain (Stratagene, La Jolla, Calif.) that have been modified by the addition of a genetic construction for the expression of alpha-amylase isolated from a thermophilic bacteria and have the ATCC accession numbers 69480, 69481 and 69482, respectively. The plasmid containing the alpha-amylase gene in the E. coli strains having ATCC accession numbers 69480, 69481 and 69482 may be readily transferred to other strains of bacteria using techniques well known to the person of average skill in the art. Similarly, the person of average skill in the art may excise the alpha amylase gene from plasmids in the E. coli strains having accession numbers 69480, 69481 and 69482 and transfer the alpha amylase gene to a new genetic construct prior to transferring the gene to a new strain of bacteria.

Detailed Description Text - DETX (6):

An alpha-amylase gene from a recently isolated uncharacterized thermophilic bacterium was initially inserted onto an autonomously replicating plasmid DNA element, and then was introduced into several E. coli strains through conjugation. This alpha-amylase gene can be found in the plasmids present in the E. coli strains having the ATCC accession numbers 69480, 69481, and 69482.

The resultant alpha-amylase gene containing strains and the respective parent strain were rendered competent using the procedure of Hanahan (J. Mol. Biol. (1983)). The transformation efficiency of the alpha-amylase gene containing strains were compared with the transformation of similar strains lacking the alpha-amylase gene.

Detailed Description Text - DETX (11):

Cloning alpha-amylase gene onto RSF1010 derivative pAL205. pBM100, a pBluescript.TM. II vector derivative containing the amylase gene from a thermophilic bacterium was digested with XbaI and HindIII and the appropriate DNA fragment was isolated and ligated to pAL205 digested with these same two enzymes. The ligation mix was transformed into SCS 1 and Tet.sup.R Cam.sup.R colonies selected. Plasmid DNA from these transformants was isolated and subjected to restriction enzyme analysis to confirm the presence of the amylase gene.

Claims Text - CLTX (4):

2. The method of claim 1, wherein the alpha-amylase gene is isolated from a thermophilic bacterium.

Claims Text - CLTX (15):

10. The competent bacterial cell according to claim 9, wherein the alpha-amylase gene is isolated from a thermophilic bacterium.

Other Reference Publication - OREF (4):

Tsukagoshi et al., 1984, "Cloning and Expression of a Thermophilic .alpha.-Amylase Gene from Bacillus stearothermophilus in Escherichia coli," Mol. Gen. Genet. 193:58-63.

US-PAT-NO: 5665869

DOCUMENT-IDENTIFIER: US 5665869 A

TITLE: Method for the rapid removal of protoporphyrin from
protoporphyrin IX-containing solutions of hemoglobin

DATE-ISSUED: September 9, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ryland; James R.	Louisville	CO	N/A	N/A
Matthews; Maura-Ann H.	Boulder	CO	N/A	N/A
Ernst; Ulrich P.	Lafayette	CO	N/A	N/A
Houk; Daniel E.	Concord	CA	N/A	N/A
Traylor; David W.	Wheat Ridge	CO	N/A	N/A
Williams; Lee R.	Concord	CA	N/A	N/A

APPL-NO: 08/ 153071

DATE FILED: November 15, 1993

US-CL-CURRENT: 530/412, 530/385

ABSTRACT:

The present invention relates to a method for the production of a substantially protoporphyrin IX free hemoglobin solution comprising: rapidly heating a crude protoporphyrin IX-containing hemoglobin solution for a relatively short time and at a relatively high temperature to reduce protoporphyrin IX-containing hemoglobin to insignificant levels in said protoporphyrin IX-containing hemoglobin solution.

38 Claims, 12 Drawing figures

Exemplary Claim Number: 38

Number of Drawing Sheets: 12

----- KWIC -----

Other Reference Publication - OREF (18):

Tsukagoshi, N. et al/Cloning and Expression of a **Thermophilic**
.alpha.-Amylase Gene from *Bacillus stearothermophilus* in *Escherichia coli*/Mol.
Gen. Genet./(1984)/193, 58-63.

US-PAT-NO: 5512468

DOCUMENT-IDENTIFIER: US 5512468 A

See image for Certificate of Correction

TITLE: Process of producing highly transformable bacterial
cells and cells produced thereby

DATE-ISSUED: April 30, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Greener; Alan L.	San Diego	CA	N/A	N/A

APPL-NO: 08/ 151577

DATE FILED: November 22, 1993

US-CL-CURRENT: 435/488, 435/252.33 , 435/252.8

ABSTRACT:

The invention provided herein includes gram negative bacteria cells containing a gene encoding an enzyme with carbohydrate degrading activity that had been rendered competent to transformation. Carbohydrate degrading enzymes of interest for use in the invention include alpha-amylase. The competent cells of the subject invention may be frozen so as to provide for prolonged storage.

Other aspects of the invention include methods for rendering gram negative bacterial cells, such as E. coli cells competent to transformation. These methods involve the step of transferring a gene encoding an enzyme with carbohydrate degrading activity into E. coli cells and subsequently rendering the cells competent using any of a variety of competency inducing procedures.

11 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX (14):

The term "starch-degrading enzyme" as used herein refers to enzymes capable of hydrolyzing at least one type of linkage present between the constituent monosaccharide units of a carbohydrate molecule. The term "starch-degrading enzyme" as used herein refers to enzymes capable of hydrolyzing at least one type of linkage present between the constituent monosaccharide units of a starch molecule. The term "alpha-amylase" as used herein refers to enzymes

capable of catalyzing hydrolysis of the α -1,4 glucosidic linkages of polysaccharides containing such glucosidic linkages such as starch or glycogen. Preferred carbohydrate degrading enzymes are starch degrading enzymes. Preferred starch degrading enzymes are alpha-amylases. Particularly preferred starch degrading enzymes for use in the invention are alpha-amylases isolated from thermophilic bacteria, especially the alpha-amylase gene from a recently isolated uncharacterized thermophilic bacterium. The polynucleotide sequence encoding this alpha-amylase from the uncharacterized thermophilic bacterium can be found on the FAMY plasmid present in the E. coli strains having the ATCC accession numbers 69480, 69481, and 69482.

Brief Summary Text - BSTX (18):

The introduction of a genetic construction for the expression of a carbohydrate degrading enzyme into E. coli serves to increase the transformation efficiency of compositions of E. coli cells rendered competent for a wide variety of E. coli strains. The genotype of an E. coli cell strain containing a genetic construction for the expression of a carbohydrate degrading enzyme may be selected so as to be particularly useful for a given genetic engineering experiment. For example, cloning vectors that are screenable because of LacZ.alpha. fragment complementation may contain a particular mutation within the LacZ gene. Similarly, the cell may contain various other deletions or mutations in order to provide for complementation by the transforming DNA. The host cell may either possess or lack a restriction-modification system in order to expedite cloning. The host cells may also lack one or more recombination systems, e.g., RecA, RecBC. Preferred E. coli strains for use in the invention are cells that contain a mutation in the deoR gene, as described in Hanahan, U.S. Pat. No. 4,851,348. Particularly preferred strains of E. coli for use in the invention are the XL1-Blue.TM. strain (Stratagene, La Jolla, Calif.), the XL1-Blue MR strain, and the SURE.TM. strain (Stratagene, La Jolla, Calif.) that have been modified by the addition of a genetic construction for the expression of alpha-amylase isolated from a thermophilic bacteria and have the ATCC accession numbers 69480, 69481 and 69482, respectively. The plasmid containing the alphaamylase gene in the E. coli strains having ATCC accession numbers 69480, 69481 and 69482 may be readily transferred to other strains of bacteria using techniques well known to the person of average skill in the art. Similarly, the person of average skill in the art may excise the alpha amylase gene from plasmids in the E. coli strains having accession numbers 69480, 69481 and 69482 and transfer the alpha amylase gene to a new genetic construct prior to transferring the gene to a new strain of bacteria.

Detailed Description Text - DETX (5):

An alpha-amylase gene from a recently isolated uncharacterized thermophilic bacterium was initially inserted onto an autonomously replicating plasmid DNA element, and then was introduced into several E. coli strains through conjugation. This alpha-amylase gene can be found in the plasmids present in the E. coli strains having the ATCC accession numbers 69480, 69481, and 69482. The resultant alpha-amylase gene containing strains and the respective parent strain were rendered competent using the procedure of Hanahan (J. Mol. Biol. (1983)). The transformation efficiency of the alpha-amylase gene containing

strains were compared with the transformation of similar strains lacking the alpha-amylase gene.

US-PAT-NO: 5366883

DOCUMENT-IDENTIFIER: US 5366883 A

TITLE: .alpha.-amylase gene

DATE-ISSUED: November 22, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Asada; Kiyozo	Shiga	N/A	N/A	JP
Uemori; Takashi	Shiga	N/A	N/A	JP
Mukai; Hiroyuki	Shiga	N/A	N/A	JP
Kato; Ikunoshin	Kyoto	N/A	N/A	JP
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APPL-NO: 07/ 894212

DATE FILED: June 9, 1992

US-CL-CURRENT: 435/202, 435/252.3 , 435/252.31 , 435/252.33 , 435/320.1
, 435/69.1 , 435/71.2 , 536/23.1 , 536/23.2

ABSTRACT:

The present invention relates, in general, to a cloned .alpha.-amylase gene,
and, in particular, to a cloned hyperthermophilic .alpha.-amylase gene and to
methods of producing .alpha.-amylase using same.

13 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

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Abstract Text - ABTX (1):

The present invention relates, in general, to a cloned .alpha.-amylase gene,
and, in particular, to a cloned hyperthermophilic .alpha.-amylase gene and to
methods of producing .alpha.-amylase using same.

Brief Summary Text - BSTX (2):

The present invention relates, in general, to a cloned .alpha.-amylase gene,
and, in particular, to a cloned hyperthermophilic .alpha.-amylase gene and to

methods of producing .alpha.-amylase using same.

Brief Summary Text - BSTX (7):

Using genetic engineering technology, it is theoretically possible to clone genes and produce the enzymes that they encode in quantities sufficient for industrial application. A number of genes coding for thermophilic .alpha.-amylases have been isolated and subsequently expressed in *E. coli* and *B. subtilis* (Fukusumi et al, Eur. J. Biochem., 98: 95 (1985), Tsukagoshi et al, Mol. Gen. Genet., 195: 58 (1984), Tsukagoshi et al, J. Bacteriology, 164: 1182 (1985)). The temperature at which the genes are endogenously translated does not seem to have an effect on the expression in transformation competent cells. Thus it is possible to produce thermophilic enzymes in host cells grown at ambient temperature. However, no genes coding for hyperthermophilic .alpha.-amylases have ever been successfully cloned.

Brief Summary Text - BSTX (9):

The present invention provides, for the first, time a cloned sequence encoding a hyperthermophilic .alpha.-amylase. The availability of this sequence makes possible the industrial scale production of this enzyme.

Brief Summary Text - BSTX (11):

The present invention relates to an isolated DNA segment having a nucleotide sequence that encodes .alpha.-amylase, specifically, a hyperthermophilic .alpha.-amylase. The invention further relates to a recombinant method of producing hyperthermophilic .alpha.-amylase. The invention also relates to an expression vector suitable for use in such a method.

Brief Summary Text - BSTX (12):

It is a general object of the invention to provide a gene encoding a hyperthermophilic .alpha.-amylase.

Detailed Description Text - DETX (6):

The Examples that follow make reference to the hyperthermophilic bacteria, *P. furiosus* (deposited at Deutsche Sammlung von Mikroorganismen with the identification number of DSM 3638). The procedures for cloning the .alpha.-amylase gene from this bacteria and for preparing transformants carrying the gene, can be described in general terms as follows:

US-PAT-NO: 5364782

DOCUMENT-IDENTIFIER: US 5364782 A

TITLE: Mutant microbial .alpha.-amylases with increased
thermal, acid and/or alkaline stability

DATE-ISSUED: November 15, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Quax; Wilhelmus J.	Voorschoten	N/A	N/A	NL
Laroche; Yves	Brussels	N/A	N/A	BE
Vollebregt; Adrianus W. H.	Naaldwijk	N/A	N/A	NL
Stanssens; Patrick	St. Denijs Westrem	N/A	N/A	BE
Lauwereys; Marc	Haaltert	N/A	N/A	BE

APPL-NO: 07/ 623953

DATE FILED: November 29, 1990

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	89201735	June 29, 1989

PCT-DATA:

APPL-NO: PCT/EP90/01042
DATE-FILED: June 27, 1990
PUB-NO: WO91/00353
PUB-DATE: Jan 10, 1991
371-DATE: Dec 2, 1990
102(E)-DATE: Dec 2, 1990

US-CL-CURRENT: 435/202, 435/252.3 , 435/263 , 435/275 , 435/320.1 , 536/23.2

ABSTRACT:

Thermostable and acid stable .alpha.-amylases are provided as expression products of genetically engineered .alpha.-amylase genes isolated from microorganisms, preferably belonging to the class of Bacilli. Both chemical and enzymatic mutagenesis methods are e.g. the bisulphite method and enzymatic misincorporation on gapped heteroduplex DNA. The mutant .alpha.-amylases have superior properties, e.g. improved thermostability over a broad pH range, for industrial application in starch processing and textile desizing.

6 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

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Other Reference Publication - OREF (1):

Gray et al., Structural **Genes Encoding the Thermophilic alpha-Amylases** of
Bacillus stearothermophilus and Bacillus licheniformis, J. Bacteriol. (1986)
166:635-643.

US-PAT-NO: 4946789

DOCUMENT-IDENTIFIER: US 4946789 A

TITLE: Bacillus brevis strains and application thereof

DATE-ISSUED: August 7, 1990

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Udaka; Shigezo	Aichi	N/A	N/A	JP
Takagi; Hiroaki	Chiba	N/A	N/A	JP
Kadowaki; Kiyoshi	Chiba	N/A	N/A	JP

APPL-NO: 07/ 043459

DATE FILED: April 28, 1987

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	61-198120	August 26, 1986

US-CL-CURRENT: 435/252.3, 435/252.31, 435/69.1, 435/71.1, 435/71.2

ABSTRACT:

Bacillus brevis strains which produce a large amount of protein but no protease out of the cells are disclosed. These strains are highly useful as hosts in genetic engineering.

2 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

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Detailed Description Text - DETX (24):

Cloning of Thermophilic .alpha.-amylase Gene into E. Coli

Detailed Description Text - DETX (27):

SUBCOLONING OF THERMOPHILIC .alpha.-AMYLASE GENE

US-PAT-NO: 4493893

DOCUMENT-IDENTIFIER: US 4493893 A

TITLE: Process for cloning the gene coding for a thermostable
alpha-amylase into Escherichia coli and Bacillus subtilis

DATE-ISSUED: January 15, 1985

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mielenz; Jonathan R.	LaGrange Park	IL	N/A	N/A
Mickel; Susan	LaGrange Park	IL	N/A	N/A

APPL-NO: 06/ 472646

DATE FILED: March 11, 1983

PARENT-CASE:

This application is a continuation of application Ser. No. 225,287 filed
Jan. 15, 1981, now abandoned.

US-CL-CURRENT: 435/91.41, 435/201 , 435/320.1 , 435/69.1 , 435/69.2

ABSTRACT:

An improved process for producing a thermostable alpha-amylase enzyme is described. The gene coding for the alpha-amylase is incorporated into a chimeric plasmid which is produced in multiple copies by a host microorganism.

22 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (22):

The chimeric plasmids of this invention are prepared using DNA from a naturally-occurring donor microorganism which contains a **gene coding for a thermostable alpha-amylase** enzyme. Suitable donor microorganisms are found in the **thermophilic** bacteria classified as Bacillus stearothermophilus (abbreviated B. stearothermophilus) and Thermus flavus (abbreviated T. flavus). Strains of Bacillus licheniformis (abbreviated B. licheniformis) are also suitable donor microorganisms. Strains of B. stearothermophilus particularly suitable for the use as a source of donor DNA are those strains selected from the group consisting of B. stearothermophilus, ATCC Nos. 31,195; 31,196; 31,197; 31,198; 31,199 and 31,783, variants and mutants thereof and submutants

of said mutants.